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Note on rare knot worm, *Polygordius madrasensis* Aiyar and Alikunhi, 1944 (Polychaeta, Polygordiidae) from India

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Abstract

Polygordius species is a clade of smooth bodied marine annelids, which are the main inhabitants of sandy intertidal sediments. They are well adapted for life in interstitial habitats. They have a habit of tying themselves in knots when collected, giving rise to the common name "knot worm". Due to comparative morphological appearances among *Polygordius* species and its resemblance with nematodes and nemerteans made its taxonomy more challenging and required more advanced microscopic and molecular techniques. Only two species and a subspecies, primarily restricted to the south coast, have been recorded from Indian waters. This study mainly discusses the Indian species, with additional information on *Polygordius madrasensis* Aiyar and Alikunhi, 1944, based on Scanning Electron Microscopy (SEM) results.

Keywords: Interstitial, Intertidal, Kerala, Marine Annelids, Meiofauna

Introduction

The members of the genus *Polygordius* Schneider, 1868, are elongated and cylindrical interstitial marine annelids with indistinct external segmentations. This is the only genus described under the family Polygordiidae Czerniavsky, 1881. Since this group is devoid of basic annelid characters, they were once considered common ancestors of the phylum Annelida and were named Archiannelids (Worsaae, *et al.*, 2021). They have been recorded in all oceans except the Arctic Ocean. Eighteen valid species and two subspecies have been described from the intertidal zone to the continental slope region (Tustison, *et al.*, 2020). Among them, two species and a subspecies have been described from Indian waters. *P. madrasensis* and *P. uroviridis* (Aiyar and Alikunhi, 1944) were the first species to be described from Indian waters. Later, a subspecies, *P. eschaturus brevipapillosus* (Jouin and Rao, 1987) was also described from the Andaman Sea. Since the type description of *P. madrasensis* was devoid of photographs and SEM images, the present study aims to provide more information on its morphology using SEM techniques.

Material and Methods

Specimens of *P. madrasensis* were collected from the intertidal sediments of Karumkulam beach, southwest coast of India (08°32'42.2"N 77°05'60.8"E) as a part of a meiofaunal diversity study conducted on 2nd June 2019 (Figure 1). Sediment samples were collected ~ 4m away from the low tide mark towards the shore using a 3.5 cm diameter PVC corer after digging a pit in the beach sediments at a depth of 60 cm. Top 5 cm sediment layer was collected and immediately fixed in 5% buffered formalin. Initial sorting and counting of specimens were carried out using a stereo zoom microscope (Magnus MSZ-TR) and further observed under a Leica DM6 epifluorescence microscope for species identification. Morphometry was acquired using Leica Application Suite X (LAS X) using the standard method (Ramey, *et al.*, 2006). Five specimens were dehydrated in a graded ethanol series (20 minutes in each of 70, 80, 90 and 100 %), and then subsequently placed in 3 ml of Hexamethyldisilazane (HMDS) for 10 minutes to remove the ethanol. The remaining HMDS was removed and the specimen was left in a desiccator to air dry at room temperature for 18 hrs (modified

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from Murtey and Ramasamy, 2016). Dried specimens mounted on aluminum stubs were sputter-coated with gold and observed under a scanning electron microscope (VEGA3 TESCAN SEM). Neotype (voucher no: MB/SBN/VD8) was deposited at the Marine Biology Museum of the Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology (CUSAT), India. Water quality parameters were recorded using a multi-parameter waterproof meter (Make: HANNA, Model: HI98194). Dissolved inorganic nutrients were estimated by standard methods (Grasshoff, *et al.*, 1999). Sediment organic carbon and nitrogen content were determined using an ECS 4010, CHNSO analyser (Make: Costech).

Results

Distribution of genus Polygordius from Indian waters: After the first observation of *P. madrasensis* and *P. uroviridis* by Aiyar and Alikunhi, 1944 from Tamil Nadu coast, they were further recorded from a few other locations on the Indian subcontinent and its islands. Their occurrence from the east coast of India were recorded from Bhimilipatanam (Sarma and Mohan, 1981), Vishakapattanam coast (Rao and Ganapati, 1968), further (Sugumaran and Padmasai, 2019) from Manamelkudi of Palk Bay and (Altaff, *et al.*, 2005) from Marina beach followed by (Kalyani 198,8) Pulicat Lake. On the west coast of India, Rajan and Nair (1979) recorded their occurrence from Neendakara beach and later from Lakshadweep waters (Ghosh, 1991) followed by Priyalakshmi (2018) from south Kerala. Whereas, *P. eschaturus brevipapillosus* was not reported anywhere other than its type description. The general distribution of all the Indian species are shown in (Figure 1).

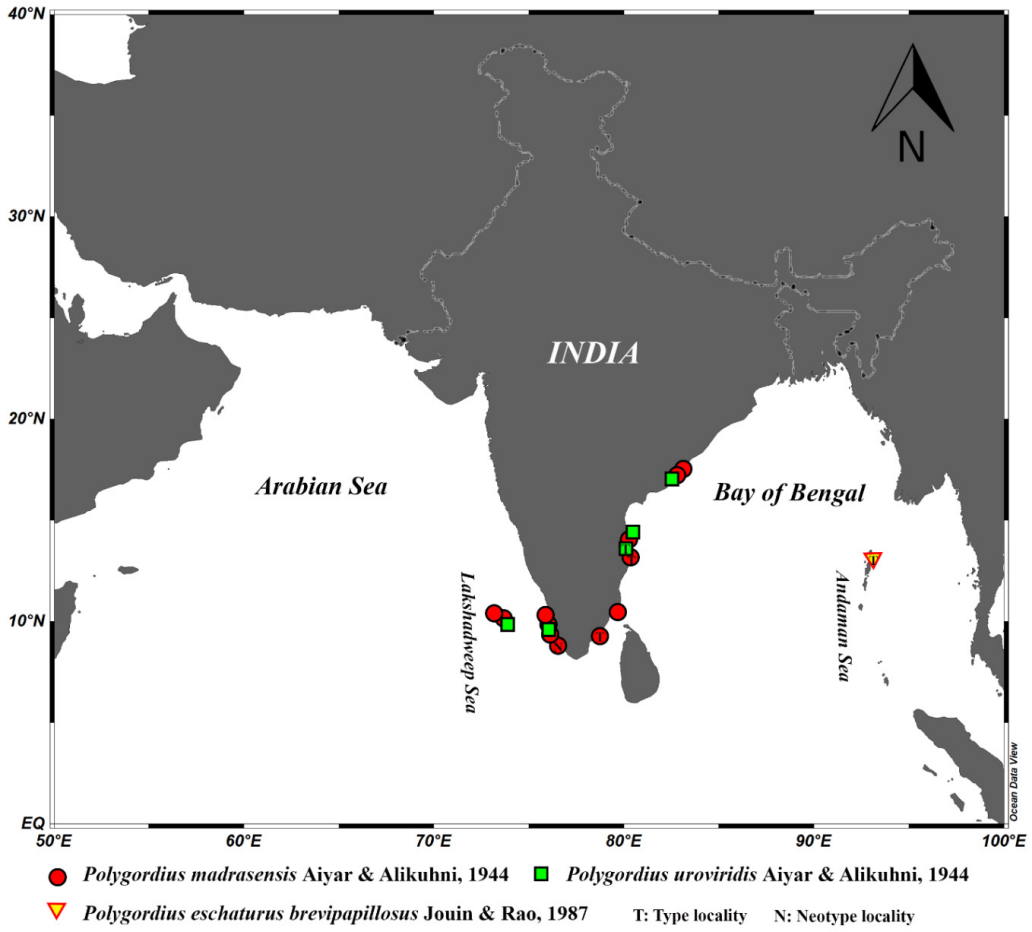

Figure 1. Map showing records of *Polygordius* species from Indian waters.

Systematics

Family POLYGORDIIDAE Czerniavsky, 1881
Genus ***Polygordius*** Schneider, 1868
Polygordius madrasensis Aiyar and Alikunhi, 1944 (Emended)
Figures 2, 3 and Table 1.
Neotype: MB/SBN/VD8

Diagnosis: Elongated, smooth and slender body with cylindrical shape; uniform width all along the body; prostomium rounded; external ciliation and segmentation absent; paired palps twice the length of the prostomium; eyespot absent; inflated pygidium bearing a pair of terminal pygidial cirri; pygidial cirri shorter than palps; well-distinct longitudinal pygidial glands (14-16); anal lobes, parapodia and chaetae are absent.

Description: The body length (from the anterior rim of the prostomium to the base of the pygidial cirri) of the neotype is 5.08 mm with a 0.11 mm width (Figure 2 E). Prostomium rounded (0.058 mm) with a pair of long un-ciliated frontally orientated palps (0.108 mm) attached closer at its anterior margin (Figure 2 A). Palp

Table 1. Morphometrics of *Polygordius* species described from Indian waters. (I), interpretation from figures; n.d - not defined

Character	*P. madrasensis* Aiyar and Alikunhi, 1944	*P. uroviridis* Aiyar andAlikuhni, 1944	*P. eschaturus brevipapillosus* Jouin and Rao, 1987	*P. madrasensis* (Neotype)	*P. madrasensis* Additional specimens examined (n = 15)
Prostomium shape	Conical (I)	Conical (I)	n.d	rounded	rounded
Palp length (mm)	0.15	n.d	0.2-0.25	0.108	0.09-0.12
Prostomium length (mm)	n.d	n.d	n.d	0.058	0.04-0.08
Antenna to prostomium ratio	1:1 (I)	1:1 (I)	n.d	1.86:1	1.4:1-1.9:1
Pigment spots (no.)	absent	present/2, rarely 4	absent	absent	absent
Headfold	n.d	n.d	n.d	shallow	shallow
Mouth shape	triangular	n.d	n.d	round	round
Pygidium shape/ width (mm)	swollen/ 0.15	swollen/ 0.12	bulb/ 0.53	inflated/ 0.132	0.1-0.15
Pygidial glands (no.)	14 -18	n.d	50-70	14	14-16
Pygidial gland shape	rod	n.d	rod	oval	oval
Glandular belt width (µm)	n.d	n.d	108	12.18	9-12
Gland width (µm)	n.d	n.d	n.d	8	8.6-9.5
Pygidial cirri length (mm) /position/no	0.08/terminal/2	absent	0.03/ terminal/2	0.05/terminal/2	0.05- 0.09
Anal lobes (no.)	absent	absent	6-8	absent	absent
Body length (mm)	7-10, rarely 15	4-6	30-40, rarely 50	5.08	0.7-5.2
Body width (mm)	0.18	n.d	0.25-0.5	0.11	0.08-0.11
Segment (no.)	40-60, rarely 75	25-35	120-160, rarely 205	nd	nd
Type locality	Madras beach and Krusadai Island	Madras beach	Sound Island, Andamans	Karumkulam, Kerala	Karumkulam, Kerala
Sediment type	sandy	sandy	coarse sand	coarse sand	coarse sand
Depth range (cm)	n.d	n.d	5	60	60

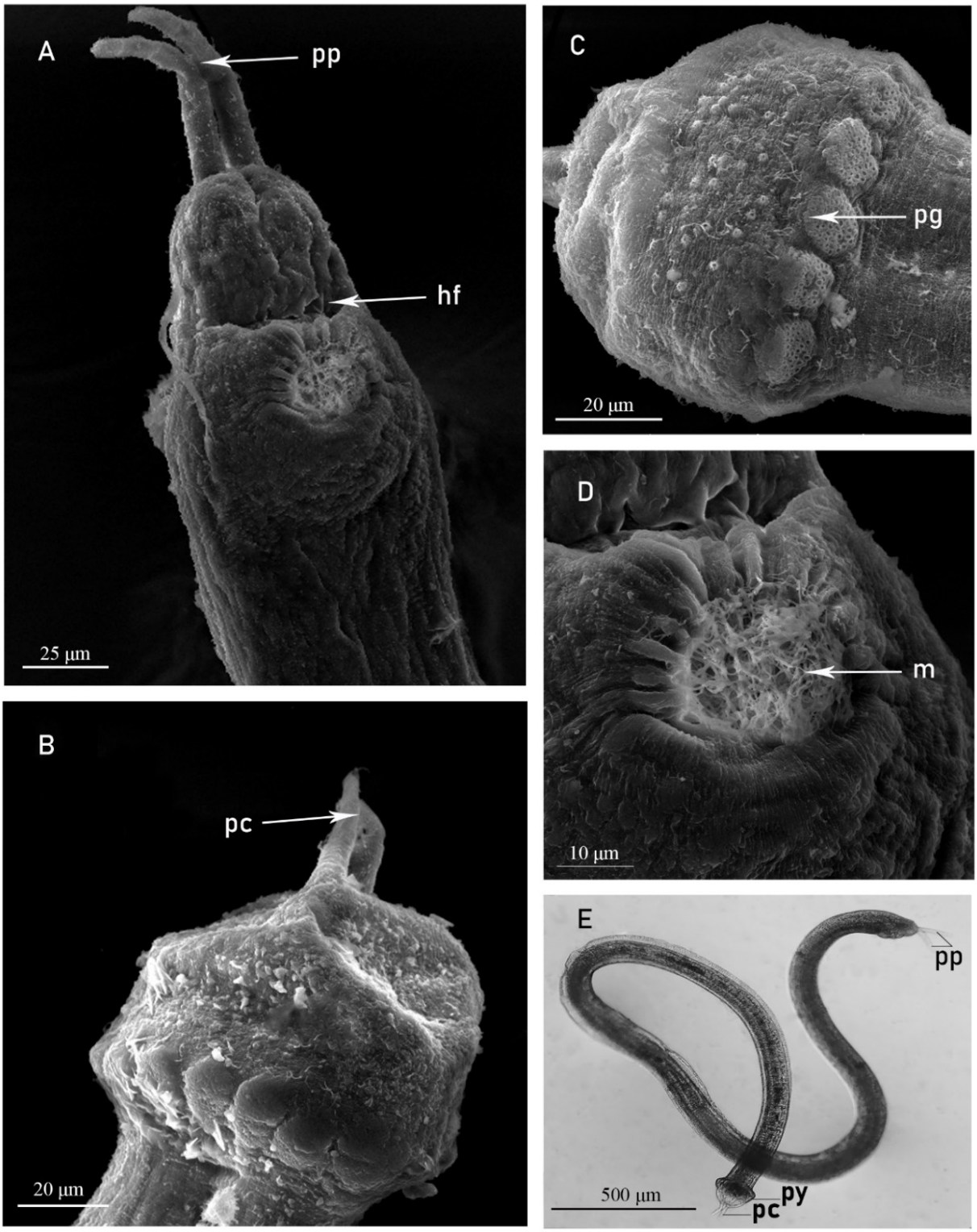

Figure 2. SEM images of *Polygordius madrasensis*: **A.** Ventral view of prostomium showing head fold and paired palps; **B.** lateral view of posterior end/pygidium with cirri; **C.** posterior end showing pygidial glands; **D.** ventral view of prostomium showing mouth; **E.** light microscopy image of habitus (pp: paired palps, hf: head fold, pc: pygidial cirri, pg: pygidial glands, m: mouth, py: pygidium).

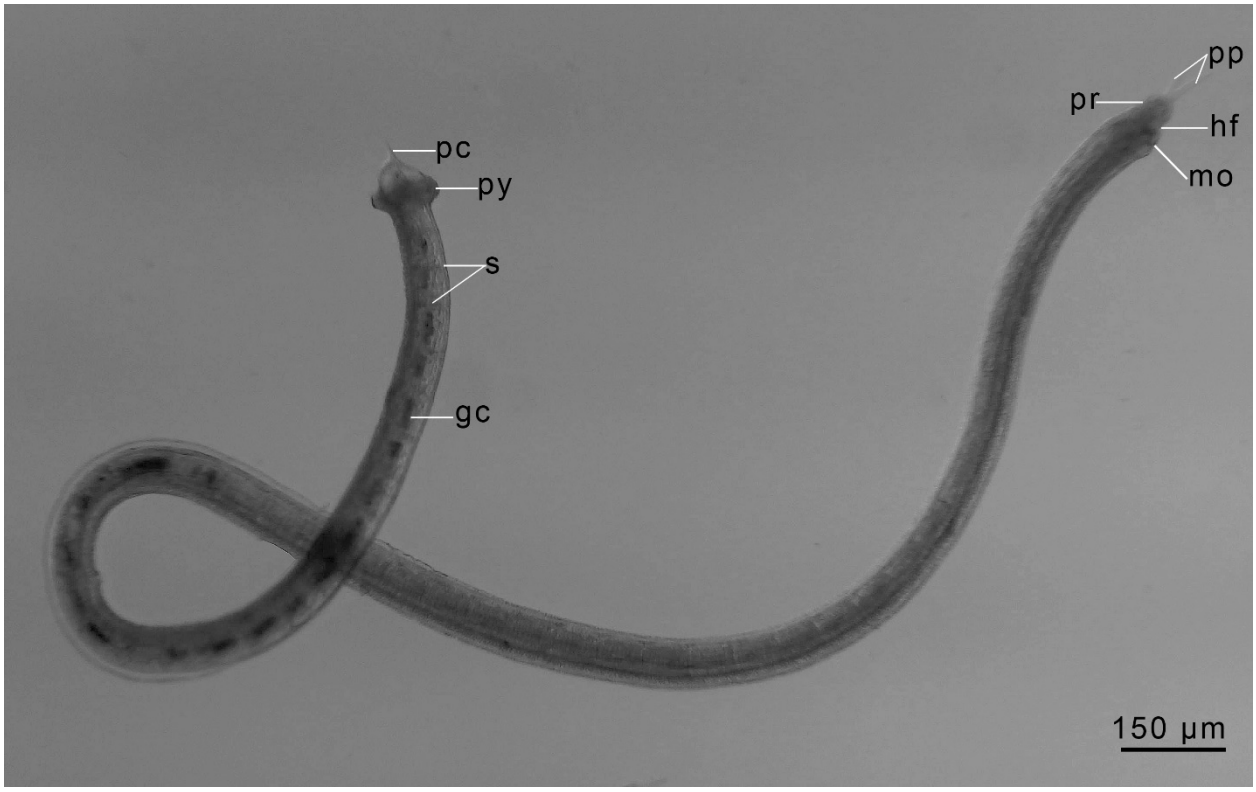

Figure 3. Light microscopy image of neotype (MB/SBN/VD8) (pp: paired palps, hf: head fold, pr: prostomium, mo: mouth opening, pc: pygidial cirri, py: pygidium, s: segments, gc: gut content).

to prostomium ratio, 1.86:1. Shallow head folds separate the prostomium and peristomium (Figure 2 A). Pigment spots are absent. Circular mouth (30 µm) positioned ventrally opens into a highly ciliated cavity (Figure 2 D). The posterior region consists of an inflated pygidium with a width of 0.132 mm and a pair of terminal pygidial cirri (0.054 mm) (Figure 2 B). A glandular belt (12.18 µm) is present at the base of the pygidium. 14-16 highly distinct, oval shaped and longitudinally oriented pygidial glands (av. 12 µm length and 8 µm width) are present with a space between them (Figure 2 C). Terminal anus with no anal lobes. The body is devoid of parapodia and chaetae (Figure 3).

Ecology: Salinity and temperature of the interstitial water from the sampling site were recorded as 35.2 PSU and 30 °C respectively, with a pH of 8.06. Porewater dissolved oxygen was recorded as 3.24 mg/L. The porewater dissolved inorganic nitrogen and dissolved inorganic phosphorus were 12.41 µmol/L and 1.71 µmol/L respectively. Whereas dissolved inorganic silicate was recorded as 2.4 µmol/L. Sediment type was sandy. The sediment organic carbon percentage was noted as 0.29% while total nitrogen was near to 0.003%. *P. madrasensis* showed a numerical abundance of 149 ind/10 cm^2.

Discussion

All the *Polygordius* species from India were described from sandy intertidal regions. Global research has also revealed that, *Polygordius* have more affinity towards dynamic environments with coarse sediments (Worsaae, *et al.,* 2021). Adaptation to life in an interstitial habitat has given them a long and slender body type. Among the Indian species, *P. eschaturus brevipapillosus* possess higher body length and segmentation than *P. madrasensis* and *P. uroviridis*. Paired palps, which lack ciliation of varying length functions as sensory receptor (Tustison, *et al.,* 2020). Pigment spots are only present in *P. eschaturus brevipapillosus*. Pygidial glands are underdeveloped in *P. uroviridis,* while *P. madrasensis* and *P. eschaturus brevipapillosus* consist of longitudinally oriented 14-18 and 50-70 glands respectively. Pygidial glands of varying size, shape, and number in different species acts as an adhesive function in the sediments. Pygidial cirri are

absent in *P. uroviridis,* whereas the other two congeners possess a pair of terminal cirri. None of the Indian species possess ciliated pygidium, parapodia and chaetae. The sexes are separate. Anal lobes are only present in *P. eschaturus brevipapillosus.* Comparative morphometrics of all the Indian species are displayed in Table 1.

All the Indian species were recorded from sediments with high porewater salinity (Aiyar and Alikuhni, 1944; Jouin and Rao, 1987). Higher organic carbon loads in sandy sediments with high porewater salinity and oxygen saturation might be the reason behind its mass occurrence (Ramey, 2008). Observations on *P. jouinae* Ramey, Fiege and Leander, 2006 have also found that *Polygordius* species normally prefer organic carbon rich and well oxygenated environments (Worsaae, *et al.,* 2021). *P. triestinus* was the only species reported from oxygen deficient, muddy sediments (Hempelmann, 1906).

Despite the fact that there are numerous records of *P. madrasensis* from Indian waters, no studies have attempted a taxonomic reinvestigation using modern techniques. This species was taxonomically unexplored for eighty years. The specimens of *P. madrasensis* examined in the present study were found to be shorter and smaller than the type specimen. A conical-shaped prostomium was recorded in the type description, whereas the current specimen possesses a round prostomium. The palp to prostomium ratio was also higher in the current specimens. The shape of the pygidial gland was oval in the present study, while it was rod shaped in the original description. The pygidial glands are one of the major taxonomic characteristics of this group and they were only visible in SEM. Since the neotype displayed a few but significant anomalies in morphology in contrast to the original description, molecular data is inevitable for the further taxonomy of this group. As there are no molecular data available for any of the Indian species, a detailed study is to be conducted on these aspects.

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References

Aiyar, R.G. and Alikunhi, K.H. 1944. On some archiannelids of the Madras coast. *Proceedings of the National Institute of Science*, India, **10**(1): 113-140.

Ghosh, A.K. 1991. *Fauna of Lakshadweep*. Published by Zoological Survey of India, Kolkata, 83-84.

Altaff, K., Sugumaran, J. and Naveed, M.S. 2005. Impact of tsunami on meiofauna of Marina beach, Chennai, India. *Current Science,* **89**(1): 34-38.

Czerniavsky, V. 1881. Material ad zoographiam Ponticam comparatam. Fasc. III Vermes. Bulletin de la Societe Impériale des naturalistes de Moscou (= Byulletin' Moskovskogo obshchestva ispytatelei prirody, **55**(4): 211-363.

Grasshoff, K., Kremling, K. and Ehrhardt, M. 1999. *Methods of seawater analysis, Third Edition.* Published by John Wiley and Sons, 632. https://doi.org/10.1002/9783527613984

Hempelmann, F. 1906. Zur Morphologie von *Polygordius lacteus* Schn.und *Polygordius triestinus* Wolterek, nov. spec. *Zeitschrift fuer Wissenschaftliche Zoologie,* **84**: 527-1868.

Jouin, C. and Rao, G.C. 1987. Morphological studies on some Polygordiidae and Saccocirridae (Polychaeta) from the Indian Ocean. *Cah. Biol. Mar*. **28**: 389-402.

Kalyani, N. 1988. The Meiofauna in and around the Pulicat Lake. Ph.D. Thesis, University of Madras, 55.

Murtey, M.D. and Ramasamy, P. 2016. Sample preparations for scanning electron microscopy–life sciences. *Modern Electron Microscopy in Physical and Life sciences*, 161-185. https://doi.org/10.5772/61720

Priyalakshmi, G. 2008. Studies on taxonomy of interstitial fauna of some prominent beaches of Kerala. Ph.D. Thesis, Cochin University of Science and Technology, 284.

Rajan, K.C. and Nair, N.B. 1979. Colonization of graded sand by the interstitial fauna of Monazite sands. *Indian journal of marine sciences*, **8**: 180-182.

Ramey, P.A. 2008. Processes affecting macrofaunal community structure in sandy sediments on the New Jersey inner continental shelf with a focus on the dominant polychaete, *Polygordius jouinae*. Ph.D. Thesis, Rutgers the State University of New Jersey-New Brunswick, 132-149.

Ramey, P.A., Fiege, D., and Leander, B.S. 2006. A new species of *Polygordius* (Polychaeta: Polygordiidae): from the inner continental shelf and in bays and harbours of the north-eastern United States. *Journal of the Marine Biological Association of the United Kingdom*, **86**(5): 1025-1034. https://doi.org/10.1017/S0025315406014007

Rao, G.C. and Ganapati, P.N. 1968. On some archiannelids from the beach sands of Waltair coast. *Proceedings of the Indian Academy of Sciences*-Section B, **67**(1): 24-30. https://doi.org/10.1007/BF03051681

Schneider, A. 1868. Ueber Bau und Entwicklung von *Polygordius*. Archiv für Anatomie, Physiologie und wissenschaftliche Medicin, 51–60.

Siva Rama Sarma, N. and Chandra Mohan, P. 1981. On the ecology of the interstitial fauna inhabiting the Bhimilipatnam coast (Bay of Bengal). Mahasagar –*Bulletin of the National Institute of Oceanography,* **14**(4): 257-263.

Sugumaran and Padmasai, R. 2019. Meiofaunal diversity and density of Manamelkudi – an intertidal sandy beach of Palk Bay, India. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences,* **5**(2): 31-46. https://doi.org/10.26479/2019.0502.03

Tustison, C.A., Ramey-Balci, P.A., and Rouse, G.W. 2020. More knot worms: Four new *Polygordius* (Annelida) species from the Pacific and Caribbean. *Diversity*, **12**(4):146. https://doi.org/10.3390/d12040146

Worsaae, K., Kerbl, A., Domenico, M.D., Gonzalez, B.C., Bekkouche, N., and Martinez, A. 2021. Interstitial Annelida. *Diversity,* **13**(2):77. https://doi.org/10.3390/d13020077